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(54) Title: METHOD FOR THE STIMULATION OF SPERM PRODUCTION AND GONADAL DEVELOPMENT IN ANIMALS

(57) Abstract

Disclosed is a method to stimulate or enhance sperm development in males and gonadal development in females by administration of an agent which results in a transiently lowered level of a thyroid hormone such as T_3 and increased gonadotropin levels in the blood.

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Method for the Stimulation of Sperm Production and Gonadal Development in Animals

Background of the Invention

Cross Reference to Related Applications

The present application claims priority to U.S. application 60/073,550, filed February 3, 1998, the contents of which are fully incorporated by reference herein.

Field of the Invention

The invention relates to method of enhancing gonadal development in an animal by administration of an agent which results in lowered levels of a thyroid hormone such as T_3 and elevation of plasma gonadotropins (luteinizing hormone (LH) and follicle stimulating hormone (FSH)).

Related Art

Over the past few decades two environmental manipulations have been used in the poultry industry to regulate the onset of sexual maturation: photoperiod and nutrition. Pullets kept in total darkness or under short photoperiods display a delay in initial egg production (Wilson and Woodward, *Poultry Sci. 37*:1054-1057 (1958); King, *Poultry Sci. 40*:479-484 (1961)). In contrast, increasing day lengths have been shown to stimulate reproductive development in many avian species including domestic fowl (Farner and Follett, *J. Anim. Sci. 25*:Suppl. 90-118 (1966); van Tienhoven and Planck, The effect of light on avian reproductive activity, Handbook of Physiology, Endocrinology II, Part 1, Chapter 4, pp. 79-107 1972). Chicks can be maintained at a physiological age of about 10 days for many months using protein-, amino acid-, or energy-deficient diets just sufficient to fill maintenance requirements (McCance, *Br. J.*

Nutr. 14:59-73 (1960); Dickerson and McCance, *Br. J. Nutr. 14*:331-338 (1960)). Return to an unrestricted, nutritional diet restores growth and development to a normal rate with little subsequent effect on adult body size or egg production (McRoberts, *J. Nutr. 87*:31-40 (1965)).

Continued manipulation of photoperiod and nutrition (particularly feed restriction) has been used in the broiler industry. The latter has become a necessary management tool due to a propensity for broiler-breeders to become obese. This is a consequence of the marked improvements in weight gain and feed conversion made by genetic selection and advances in the knowledge of dietary requirements. Unfortunately, rapid growth rate in broilers has resulted in obesity and decreased efficiency in the reproductive system of breeder stock (Reddy, Artificial insemination of broilers: economic and management implications. In:Proceedings of the First International Symposium on the Artificial Insemination of Poultry. The Poultry Science Assoc., Inc., Savoy, IL, pp.73-89, 1994). Research has been completed comparing the reproductive performance of females between broiler breeder and egg-laying strains of domestic chickens (Dunn and Sharp, *J. Reprod. Fert. 90:*329-335 (1990); Eitan and Soller, *Poultry Sci. 70*:2017-2022 (1991); *Poultry Sci. 73*:769-780 (1994); *Poultry Sci. 75*:828-832 (1996); Robinson, Ovarian form and function in chickens of varying reproductive status. Final Report, Alberta Agricultural Research Institute Project # AAR1920202. Univ. Alberta, Edmonton, Canada (1994); Eitan *et al. Poultry Sci. 77*:1593-1600 (1998)). Conclusions that have been reached from the cited studies are:

1. Female broilers compared to Leghorns are less responsive to photoperiodic manipulations with respect to optimal reproductive performance (Eitan and Soller, *Poultry Sci. 70*:2017-2022 (1991); *Poultry Sci. 73*:769-780 (1994); Eitan *et al. Poultry Sci. 77*:1593-1600 (1998));

2. Layer and broiler females differ in the minimal number of hours of light required to stimulate release of gonadotropins and initial development of their reproductive systems [critical day length, (CDL)] and the minimal number

of hours of light above which no further increase in release of gonadotropins occurs [saturation day length, (SDL)] (Dunn and Sharp, *J. Reprod. Fert. 90*:329-335 (1990); Eitan *et al. Poultry Sci. 77*:1593-1600 (1998)); and,

3. Broiler breeder hens are less responsive to artificially increased photoperiodic manipulations compared to table egg layers under the effects of feed restriction (Robinson, Ovarian form and function in chickens of varying reproductive status. Final Report, Alberta Agricultural Research Institute Project # AAR1920202. Univ. Alberta, Edmonton, Canada (1994); Eitan *et al. Poultry Sci. 77*:1593-1600 (1998)).

Eitan and Soller, *Poultry Sci. 75*:828-832 (1996), compared the performance of male broiler breeders to that of male Leghorn or layer-type poultry under controlled photoperiod and/or dietary manipulations. They developed a maturation index for comparing different lines of birds.

There are clear indications that the reproductive system of broiler breeders has been compromised, particularly during the past decade (Beaumont, *et al. Br. Poult. Sci. 33*:649-661 (1992); Reddy, Artificial insemination of broilers: economic and management implications. In:Proceedings of the First International Symposium on the Artificial Insemination of Poultry. The Poultry Science Assoc., Inc., Savoy, IL, pp.73-89 (1994); Eitan and Soller, *Poultry Sci. 75*:828-832 (1996); Goerzen *et al. Poultry Sci. 75*:962-965 (1996)). Elite male broiler breeders have been shown to exhibit premature loss of adequate numbers of viable spermatozoa. It has been suggested that up to 80% of selected males in pure lines are lost due to significant decreases in semen production (Personal communication with primary breeder personnel in the broiler industry; unpublished data from over 200 male broiler breeders). This marked reduction of selection potential can dramatically reduce genetic progress that can be made within a given type of bird, reducing the future competitiveness of specific lines.

Sulfamethazine (SMZ) is an antibiotic developed by Merck in the late 1940s for treating fowl cholera (Kiser *et al. Poultry Sci. 27*:257-262 (1948)) as well as other poultry diseases, such as coccidiosis. A side effect associated with

chronic use of SMZ is a marked red coloration of the comb and increased size of both comb and testes (van Tienhoven *et al. Poultry Sci. 35*:179-191 (1956)). Its mechanism of action in this regard is unknown. Further studies have been conducted with broiler chicks. The compound significantly increases testes development, transiently suppresses a thyroid hormone, increases plasma gonadotropins, appears to augment photoperiodic response, induces the hypothalamo-pituitary-gonadal axis, and increases the number of immuno-stained neuropeptide Y(NPY) neurons in the mediobasal hypothalamus and infundibular nucleus (IN) (Macko, Walsh and Kuenzel, *Brain Res. Bull. 44*:707-713 (1997); Kuenzel, Macko, Walsh and Proudman, *In* Perspectives in Avian Endocrinology (Eds. S. Harvey and R.J. Etches), Journal Endocrinology Ltd., Bristol, pages 81-90 (1997). In addition, it has been shown that intracerebroventricular (ICV) administration of NPY to chicks stimulates growth of the testes (Fraley and Kuenzel, *Life Sci. 52*:1649-1656 (1993)). In the rat, it has been shown that NPY neurons in the arcuate nucleus [the IN of the chick is equivalent to the arcuate n. (ARC) of mammals, (Kuenzel and van Tienhoven, *J. Comp. Neurol. 206*:292-313 (1982)), appear to be involved in augmenting the LH surge in females (Kalra and Crowley, *Ann. N.Y. Acad. Sci. 611*:273-283 (1984); Sar *et al. Endocrinology 127*:2752-2756 (1990)).

Summary of the Invention

The invention relates to a method for enhancing the development of viable sperm in a male animal and ovarian development in a female animal, comprising administering to said animal an effective amount of an agent which transiently lowers the levels of a thyroid hormone, specifically T_3, e.g. by affecting its synthesis or metabolism, and which agent also increases gonadotropins. The most robust effect occurs in males. Normally, semen is not obtained from commercial poultry lines until 16-25 weeks of age. According to

the present invention, semen production is produced by 9 weeks of age. Thus, the present invention represents a significant advance in the art.

The invention also relates to a method for synchronizing the onset of puberty in feed-restricted and light-restricted birds by administering to the birds the agent on or about the time that the photoperiod is increased (e.g. weeks 20 through 28 for broiler breeders). The invention also relates to a method for administrating the agent near the end of a bird's reproductive cycle to maintain and extend its reproductive productivity. All three applications of the invention result in a transient lowering of the level of a thyroid hormone and elevation of plasma gonadotropin levels.

The invention is also directed to a method of preparing the diets of the animals and their storage to ensure a uniform distribution and stability of the agent thereby effecting a uniform gonadal response by the animals consuming the rations.

The invention serves not only to bring animals earlier into the reproductive state. When coupled with photoperiodic manipulation, the agent can be withdrawn from the diet and the reproductive state can be maintained by photostimulation with a long, daily photoperiod.

In addition, the present invention overcomes the problem of early cessation of the reproductive systems of animals. In the poultry industry, some males exhibit a collapse of their reproductive system earlier than expected. The result is a significant decrease in the fertility of eggs produced by a particular flock. According to the present invention, an agent which results in reduced levels of a thyroid hormone and elevated gonadotropin blood levels can be administered to the animal to maintain or extend the length of the viable reproduction period.

The agent also stimulates gonadal development in female poultry. Thus, the invention also relates to a method for stimulating development of the ovary of female poultry, comprising administering to said poultry an effective amount of the agent. The invention also relates to a method for maintaining egg

production of female poultry for a period of time longer than usual, comprising administering to said poultry an effective amount of the agent near the end of the reproductive stage of their life cycle.

A common practice in the poultry industry is to recycle birds for a second season. This involves inducing a molt which in turn causes regression of the gonads. The agent can be used to bring birds back into a reproductive state sooner. In addition, the agent can be used at the end of the second reproductive cycle to sustain their productivity for a longer period of time. Thus, in a method to recycle birds for a second season involving inducing a molt whereby regression of the gonads occurs, the invention also relates to the improvement comprising administering to the birds an effective amount of the agent, whereby the birds are brought back into a reproductive state sooner. The invention also relates to a method to sustain the reproductive productivity of recycled birds at the end of their second reproductive cycle, comprising administering to the birds an effective amount of the agent. The invention is not limited to chickens and has beneficial effects in turkeys, quail, guinea fowl, ducks, game birds and other avian species.

In addition to stimulating gonadal development, the compound likewise stimulates song in pet birds that have regressed gonads. Thus, the invention also relates to a method to stimulate song in a pet bird that has regressed gonads, comprising administering to the bird an effective amount of the agent.

Brief Summary of the Drawings

Fig. 1 depicts a time line showing a typical broilerization program for elite male broiler breeders, where L=light, D=dark.

Fig. 2 depicts a graph showing the effect of the "metabolic" or feed restriction phase of broilerization on testis development.

Fig. 3 depicts a graph showing the effects on both the "metabolic" and "photoperiodic"phases of broilerization on testis size, where BW=body weight (sampled from weeks 28-50).

Fig. 4 depicts a bar graph showing the testes response resulting from the "genetic selection" phase of broilerization (see Fig.1) plus 0.2% sulfamethazine (SMZ) added to a standard starter ration at one week of age.

Figs. 5A and 5B depict graphs showing weekly levels of plasma luteinizing hormone (LH; 5A) and follicle stimulating hormone (FSH; 5B) in male broiler chicks fed a diet with 0.2% SMZ beginning at one week of age (*=$p>0.05$).

Fig. 6 depicts a graph showing daily levels of LH immediately following dietary administration of 0.2% SMZ, beginning at one week of age (*=$p<0.05$).

Figs. 7A and 7B depict bar graphs showing testis weight response (7A) and plasma LH (7B) resulting from the "photoperiodic" phase of broilerization plus 0.2% SMZ added to a grower diet from 20 to 28 weeks of age. Testes and blood were sampled at 28 weeks of age. Photoperiod = LD 14:10.

Figs. 8A and 8B show plasma FSH (8A) and testosterone (8B) resulting from blood samples taken as described with respect to Figs. 7A and 7B.

Fig. 9 depicts a bar graph showing testes weight following 0.2% SMZ (1-4 weeks of age) in chicks exposed to LD 24:0 or LD 8:16.

Detailed Description of the Preferred Embodiments

The present invention relates to a method of enhancing, maintaining or stimulating the production of viable sperm in male animals and gonadal development in females. Any animal which may experience the beneficial effects of the invention may be treated according to the present invention. In general, such animals are not nocturnal and are photoperiodic. Thus, such animals must experience photoperiodic effects, e.g the elevation of blood gonadotropins and gonadal steroid levels upon the exposure to long day lengths.

In a preferred embodiment, the animal is an avian species. Alternatively, the animals are mammals, e.g. cattle, goats, sheep, horses or other veterinary animals, zoo animals, pet animals or humans.

Alternatively, the agent can be administered to stimulate the development of the female reproductive system, e.g to stimulate ovarian development.

Elite broiler breeder males, in particular, have experienced a significant decrease in reproductive performance due to their large body size. (In contrast, Leghorn males have not shown any decline in reproductive performance in recent years.) Therefore, in a preferred embodiment, the animals treated according to the present invention are elite male broiler breeders and male turkey breeders. Alternatively, the agent can treat other poultry, game bird, zoo or pet avian species that need stimulation of their reproductive system.

According to the present invention, such animals may be treated to stimulate the production of viable sperm either at an early age or at a later time, for example, after being kept in total darkness, short photoperiods, or after being fed a protein-, amino acid- or energy-deficient diet (a "marked feed-restricted" diet). In a preferred embodiment, the animals are treated with the agent and at the same time are exposed to a long photoperiod. Long photoperiods are at least LD 14:10, and preferably, LD 20:4 or greater. In contrast, short photoperiods are about 8 hours per day or less.

The light of the photoperiods may be sun light or artificially produced light. Fluorescent and incandescent light can be used. However, light which simulates the wavelength and spectrum of natural sunlight is best.

The early application of the agent may be initiated at week 1 of age and continue for about 8 to 12 weeks, preferably about 8 weeks in order to decrease generation time and obtain semen by 9-10 weeks of age (for artificial insemination of females). In a second application, the agent can be administered during the photostimulation phase (20-28 weeks) in order to synchronize the onset of puberty in males. The feed restriction phase of broilerization (weeks 8 through 20) significantly stresses the poultry. As a result, at least some of the poultry will not respond to a long day schedule of photostimulation at 20 weeks of age. Administration of the agent overcomes this problem. A third period of

administration is near the end of the reproduction phase of their life cycle in order to extend and maintain reproductive productivity (week 40 and beyond).

In the case of transgenic animals, the advantage of the invention is that it decreases generation time, particularly in the male lines. Traits of interest, e.g. heterologous proteins, may therefore be expressed more rapidly in offspring and/or the eggs. Thus, the invention also relates to a method of decreasing generation time of animals, e.g. transgenic animals, and/or the time necessary to produce transgenic eggs, comprising administering to a male animal at an early time in its life, e.g. before the time that sperm is produced naturally, an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins thereby stimulating the early production of viable sperm, and artificially inseminating female animals with the sperm. Either one or both of the male and female animals may be transgenic. Optionally, transgenic eggs may be collected and the heterologous protein harvested. It normally takes 16-25 weeks to generate viable sperm in male chickens. According to the present invention, it is possible to decrease this time to 9 weeks.

In a preferred embodiment, chicks from a broiler (elite broiler breeder) male line are raised in Petersime batteries from hatch until one week of age. At one week of age, chicks are maintained on a long-day schedule (LD 20:4) or greater and fed a diet supplemented with 0.2% SMZ to stimulate the production of viable sperm. Once stimulated, the agent may be withdrawn and the long-day photoperiod schedule maintained to ensure continued production of viable sperm.

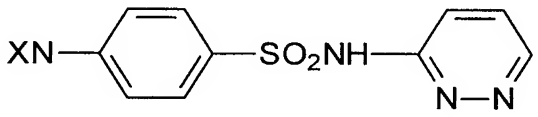
Other agents which inhibit the production *in vivo* of a thyroid hormone or affect their metabolism and which may be used in the practice of the present invention include, for example, compounds such as sulfonamides or pyrimidine sulfonamide derivatives, such as substituted 4-aminobenzenesulfonamides. Such compounds include the following:

U.S. Patent No. 3,214,335 discloses 2-sulfanilamido-5-alkoxypyrimidines.

U.S. Patent No. 2,240,496 discloses substituted N-(*p*-aminobenzene-sulfonyl)benzamide.

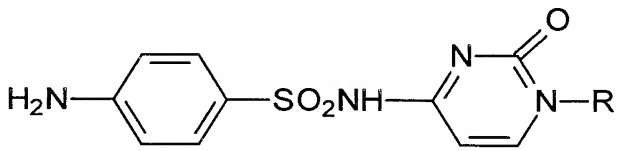
U.S. Patent No. 2,411,495 discloses 4-aminobenzenesulfonacetyl amides.

U.S. Patent No. 2,790,798 discloses substituted 4-aminobenzene-sulfonamides of the formula:



wherein X is an alkanoyl group.

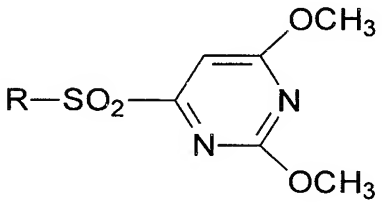
U.S. Patent No. 3,375,247 discloses substituted 4-aminobenzene-sulfonamides of the formula:



wherein R is lower alkyl, lower alkoxyalkyl, lower alkenyl or phenyl-substituted lower alkyl.

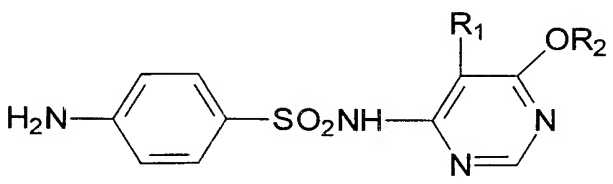
U.S. Patent No. 2,417,005 discloses *p*-aminobenzene sulfone N^1-acetylamides.

U.S. Patent No. 3,127,398 discloses substituted sulfonamides of the formula:



where R is lower alkyl, phenyl lower alkyl, phenyl, naphthyl, lower alkanoylaminophenyl, hydroxy and OMe, wherein Me represents a metal atom selected from the group consisting of alkali metal and alkaline earth metal.

U.S. Patent No. 3,132,139 discloses substituted 4-aminobenzene-sulfonamides of the formula:

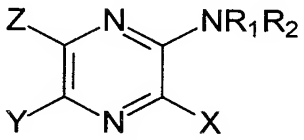


wherein R_1 and R_2 are lower alkyl groups or R_1 is H or OR_2.

U.S. Patent No. 2,218,490 discloses N-(*p*-aminobenzenesulfonyl)-benzamide.

U.S. Patent No. 3,562,258 discloses N^1-[*p*-aminobenzenesulfonyl]-N^3-[4,5-dimethyloxazolyl-(2)]guanidine.

U.S. Patent No. 3,098,069 discloses substituted 4-aminobenzenesulfonamides of the formula:



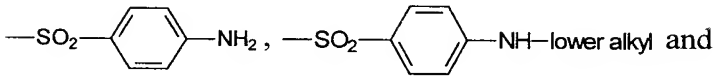
wherein X is OCH_3 or OC_2H_5;

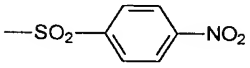
Y is H, Br or lower alkyl;

Z is H or lower alkyl;

R_1 is H or lower alkyl; and

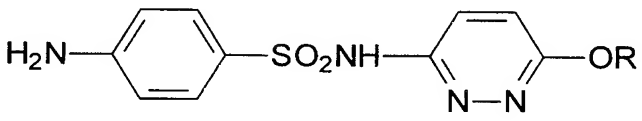
R_2 is a member of the group consisting of:





U.S. Patent No. 2,888,455 discloses 3-sulfanilamido-5-methylisoxazole.

U.S. Patent No. 2,712,012 discloses substituted 4-aminobenzenesulfonamides of the formula:



wherein R is an alkyl, aralkyl or aryl radical.

U.S. Patent No. 4,151,164 discloses 3-methoxy-4-(4'-aminobenzene-sulfonamido)-1,2,5-thiadiazole.

Preferred compounds which inhibit thyroid hormone synthesis or reduce levels of thyroid hormones (due to altered metabolism) include methimazole, thiourea, propylthiouracil, thiouracil, carbimazole, thiobarbital, and ionic inhibitors such as thiocyanate and perchlorate. Other preferred compounds include sulfathiazole, sulfaethoxypyridazine, acetyl sulfamethoxypyridazine, sulfachloro-pyrazine, mafenide, sulfisoxazole, succinylsulfathiazole, phthalylsulfathiazole, trimethoprim, sulfanilylguanidine, sulfanilamide, sulfadimindine, sulfamethylphenazole, sulfaquinoxaline, sulfapyridine, sulfapyrazine, sulfabenz, sulfabenzamide, sulfabromethazine, sulfacetamide, sulfachlorpyridazine, sulfachrysoidine, sulfacytine, sulfadiazine, sulfadicramide, sulfadimethoxine, sulfadoxine, sulfaethidole, sulfaguanidine, sulfaguanole, sulfalene, sulfaloxic acid, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethomidine, sulfamethoxazole, sulfamethoxypyridazine, sulfamethylthiazole, sulfametrole, ethylenethiourea, resorcinol and the aminoglycoside netilmicin (du Souich, P. *et al., Clin. Pharmacol. Ther. 38*:686-691 (1985)).

The agent may be administered to the animal in any means which achieves the intended purpose. For example, the agent may be administered by oral, intravenous, intramuscular, buccal, intranasal, rectal, or other means together with an acceptable carrier. In a preferred embodiment, the agent is administered to the animal as part of its food or water. In the case of humans, it is preferred that the agent be administered orally.

The dosage administered will be dependent upon the type of animal as well as the age, health, and weight, the kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. When the agent is administered as part of the food of a male broiler breeder, a preferred concentration is between about 0.1% to no more than 0.3% of the feed. In a most

preferred embodiment, the agent is sulfamethazine added at a concentration of about 0.2%.

The compositions of the invention may comprise the agent at a unit dose level of about 50 to about 600 mg/kg of body weight per day, or an equivalent amount of the pharmaceutically acceptable salt thereof, on a regimen of 1-4 times per day.

In addition to administering the compound as a raw chemical, the agent may be administered as part of a pharmaceutical preparation containing suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the compounds into preparations which can be used pharmaceutically. Preferably, the preparations, particularly those preparations which can be administered orally and which can be used for the preferred type of administration, such as tablets, dragees, and capsules, and also preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration by injection or orally, contain from about 0.01 to 99 percent, preferably from about 0.25 to 75 percent of active compound(s), together with the excipient.

The agents may be administered as a non-toxic pharmaceutically acceptable salt. Acid addition salts are formed by mixing a solution of the particular agent with a solution of a pharmaceutically acceptable non-toxic acid such as hydrochloric acid, fumaric acid, maleic acid, succinic acid, acetic acid, citric acid, tartaric acid, carbonic acid, phosphoric acid, oxalic acid, and the like. Basic salts are formed by mixing a solution of the particular agent with a solution of a pharmaceutically acceptable non-toxic base such as sodium hydroxide, potassium hydroxide, choline hydroxide, sodium carbonate and the like.

The pharmaceutical preparations of the present invention are manufactured in a manner which is itself known, for example, by means of conventional mixing, granulating, dragee-making, dissolving, or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding the

resulting mixture and processing the mixture of granules, after adding suitable auxiliaries, if desired or necessary, to obtain tablets or dragee cores.

Suitable excipients are, in particular, fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added such as the above-mentioned starches and also carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are, above all, flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated saccharide solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropymethyl-cellulose phthalate, are used. Dye stuffs or pigments may be added to the tablets or dragee coatings, for example, for identification or in order to characterize combinations of active compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the agent the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the agents are preferably dissolved or

suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of one or more of the agents with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the agents with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the agents in water-soluble form, for example, water-soluble salts and alkaline solutions. In addition, suspensions of the agents as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides or polyethylene glycol-400. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in clinical therapy and animal husbandry which are obvious to those skilled in the art are within the spirit and scope of the invention.

Examples

A management program may be employed for elite male broiler breeders (grandparent and great-grandparent stock) in order to enable geneticists to select parents for the next generation, raise them to maintain a body weight that closely matches an optimized growth curve to prevent obesity, and finally subject birds

to long-day photostimulation in order to maintain their reproductive productivity throughout their adult life. The term 'broilerization' has been used to describe this management process. Figure 1 shows a typical broilerization program for male breeders. This program is divided into three phases: the Genetic Selection Phase, Metabolic or Feed Restriction Phase and Photoperiodic/ Photostimulation Phase. The first phase incorporates a long photoperiod, and high protein/high energy starter diet in order to stimulate growth rate thereby aiding parent stock selection for the next generation. Thereafter a significantly reduced photoperiod coupled with rigorous dietary restriction is implemented to manage weight gain. This stage is referred to as the "Metabolic" Phase. Finally, birds are subjected to an increased photoperiod ("Photoperiodic" Phase) with some feed restriction to stimulate and maintain reproductive performance throughout the remainder of their life cycle. It is clear that the latter two phases of the management program are distinct and hence need to be examined separately in order to understand better the complete reproductive cycle of modern elite broiler breeders. Figure 2 illustrates how the "Metabolic" Phase compromises testes development of broilerized males. Note that the first seven weeks of feed ad libitum and long photoperiod result in stimulation of testes development. However, upon entry into the metabolic phase, severe restricted food intake and a short photoperiod (Light/Dark, LD8:16) result in a marked decline in testes size such that the gain made in testes development during the initial genetic selection phase, is lost by the end of the metabolic phase. When one examines the results of testes development obtained from a small population of elite broiler breeders using the recommended, present-day management system, much variability in testis size is seen (Fig. 3). The heterogeneous response of the testes suggests that a large proportion of the population may never develop fully functional testes.

Figure 4 shows the testes response resulting from giving a starter ration supplemented with 0.2 % SMZ during the genetic selection phase of broilerization. The compound significantly increased the rate of testes growth and by nine weeks of age, broiler chicks produced viable semen. Blood samples

were taken starting at day of SMZ administration and weekly following initiation of the treatment. Both luteinizing hormone (LH, Fig. 5A) and follicle stimulating hormone (FSH, Fig. 5B) were significantly increased. To establish how rapidly LH rises following SMZ intake, a second study showed that plasma LH was significantly elevated within 48 hr. of consuming feed containing SMZ (Fig. 6).

Sulfamethazine was also administered during the photoperiodic phase (LD 14:10) of broilerization (Weeks 20 - 28) and similar to the results obtained with one week old chicks, testis weight (Fig. 7A) of treated broiler breeders was significantly heavier than controls ($p \leq 0.05$). Blood samples were taken at week 28 and LH (Fig. 7B), FSH (Fig. 8A) and testosterone (Fig. 8B) were all significantly elevated ($p \leq 0.05$). Hence, SMZ can be administered at either a young age or near sexual maturity and development of the male gonadal system is stimulated.

In a further study using broiler chicks, SMZ at 0.2% was added to a standard broiler ration and fed to chicks from one to four weeks of age. One group was raised under continuous light while a second group was exposed to a photoperiod of LD 8:16. Results are shown in Figure 9. Chicks which consumed SMZ and were exposed to continuous light had significantly elevated testes growth ($p \leq 0.05$). In contrast, the progonadal effects of SMZ were markedly attenuated in chicks housed under a short photoperiod. Although food intake was not measured in this experiment, body weight was taken at the beginning and end of the study. No significant difference in body weight was observed between long-day and short-day SMZ treated groups, suggesting that intake of feed, and therefore SMZ, was not different between the two experimental groups. Sulfamethazine has a stimulatory effect upon testes growth when chicks are exposed to a long photoperiod. One possible interpretation is that SMZ appears to facilitate or amplify the stimulatory effect of long-day light on gonadal development in male chicks.

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those

of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention, which is defined by the following Claims.

All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those in the art to which the invention pertains. All publications, patents and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in their entirety.

What Is Claimed Is:

1. A method for enhancing the development of viable sperm in a male animal, comprising administering to said animal an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins.

2. A method for maintaining the production of viable sperm in a male animal, comprising administering to said animal an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins.

3. A method for stimulating the development of the ovary of female poultry, comprising administering to said poultry an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins.

4. The method of claim 3, wherein said poultry is near the end of its reproductive phase of its life cycle.

5. A method for maintaining egg production of female poultry for a period of time longer than usual, comprising administering to said poultry an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins.

6. In a method to recycle birds for a second season involving inducing a molt whereby regression of the gonads occurs, the improvement comprising administering to said birds an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates

gonadotropins; whereby said birds are brought back into a reproductive state sooner.

7. A method to sustain the productivity of recycled birds at the end of their second reproductive cycle, comprising administering to said birds an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins.

8. A method to stimulate song in a pet bird that has regressed gonads, comprising administering to said bird an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins.

9. A method of decreasing the generation time of an animal, comprising administering to a male animal at an early time in its life an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins thereby stimulating the early production of viable sperm in said male, and artificially inseminating a female animal with the sperm.

10. The method of claim 9, wherein at least one of said male and female animals is a transgenic animal.

11. A method of decreasing the time necessary to produce transgenic eggs, comprising administering to a male transgenic animal at an early time in its life an effective amount of an agent which inhibits transiently the production of a thyroid hormone and stimulates gonadotropins thereby stimulating the early production of viable sperm in said male, and artificially inseminating a female animal with the sperm, whereby a transgenic egg is produced, and collecting the eggs.

12. The method of any one of claims 1, 2, 9 and 11, wherein said animal is not nocturnal.

13. The method of claim 12, wherein said animal is photoperiodic.

14. The method of claim 12, wherein said animal is an avian species.

15. The method of any one of claim 1-3, 5-9 and 11, wherein said animal or bird is a chicken.

16. The method of claim 12, wherein said animal is a mammal.

17. The method of any one of claims 1-3, 5-9 and 11, wherein said agent is a substituted 4-aminobenzenesulfonamide.

18. The method of any one of claims 1-3, 5-9 and 11, wherein said agent is selected from the group consisting of methimazole, thiourea, propylthiouracil, thiouracil, carbimazole, thiobarbital, an ionic inhibitors such as thiocyanate and perchlorate.

19. The method of any one of claims 1-3, 5-9 and 11, wherein said agent is selected from the group consisting of sulfathiazole, sulfaethoxypyridazine, acetylsulfamethoxypyridazine, sulfachloro-pyrazine, mafenide, sulfisoxazole, succinylsulfathiazole, phthalylsulfathiazole, trimethoprim, sulfanilylguanidine, sulfanilamide, sulfadimindine, sulfamethylphenazole, sulfaquinoxaline, sulfapyridine, sulfapyrazine, sulfabenz, sulfabenzamide, sulfabromethazine, sulfacetamide, sulfachlorpyridazine, sulfachrysoidine, sulfacytine, sulfadiazine, sulfadicramide, sulfadimethoxine, sulfadoxine, sulfaethidole, sulfaguanidine, sulfaguanole, sulfalene, sulfaloxic acid, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole,

sulfamethomidine, sulfamethoxazole, sulfamethoxypyridazine, sulfamethylthiazole, sulfametrole, ethylenethiourea, resorcinol and netilmicin.

20. The method of any one of claims 1-3, 5-9 and 11, wherein said agent is sulfamethazine.

21. The method of any one of claims 1-3, 5-9 and 11, wherein said agent is administered as part of the food of the animal.

22. The method of claim 1, wherein said agent is administered to said animal later in its life cycle and the photoperiod is increased.

23. The method of claim 2, wherein said agent is administered to said animal at the end of its life cycle to extend its reproductive productivity.

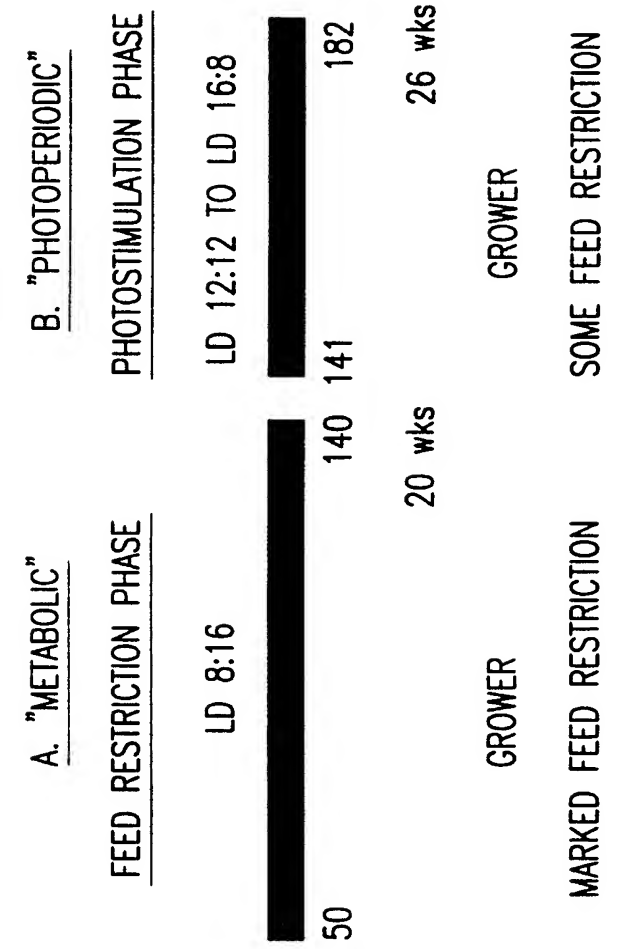

A. "METABOLIC"
B. "PHOTOPERIODIC"
GENETIC SELECTION PHASE
FEED RESTRICTION PHASE
PHOTOSTIMULATION PHASE
PHOTOPERIOD:
LD 24:0
LD 23:1
LD 8:16
LD 12:12 TO LD 16:8
DAYS:
1 2 3
49
50
140
141
182
WEEKS:
7 wks
20 wks
26 wks
DIET:
STARTER
GROWER
GROWER
MANAGEMENT:
AD LIBITUM
MARKED FEED RESTRICTION
SOME FEED RESTRICTION

FIG.1

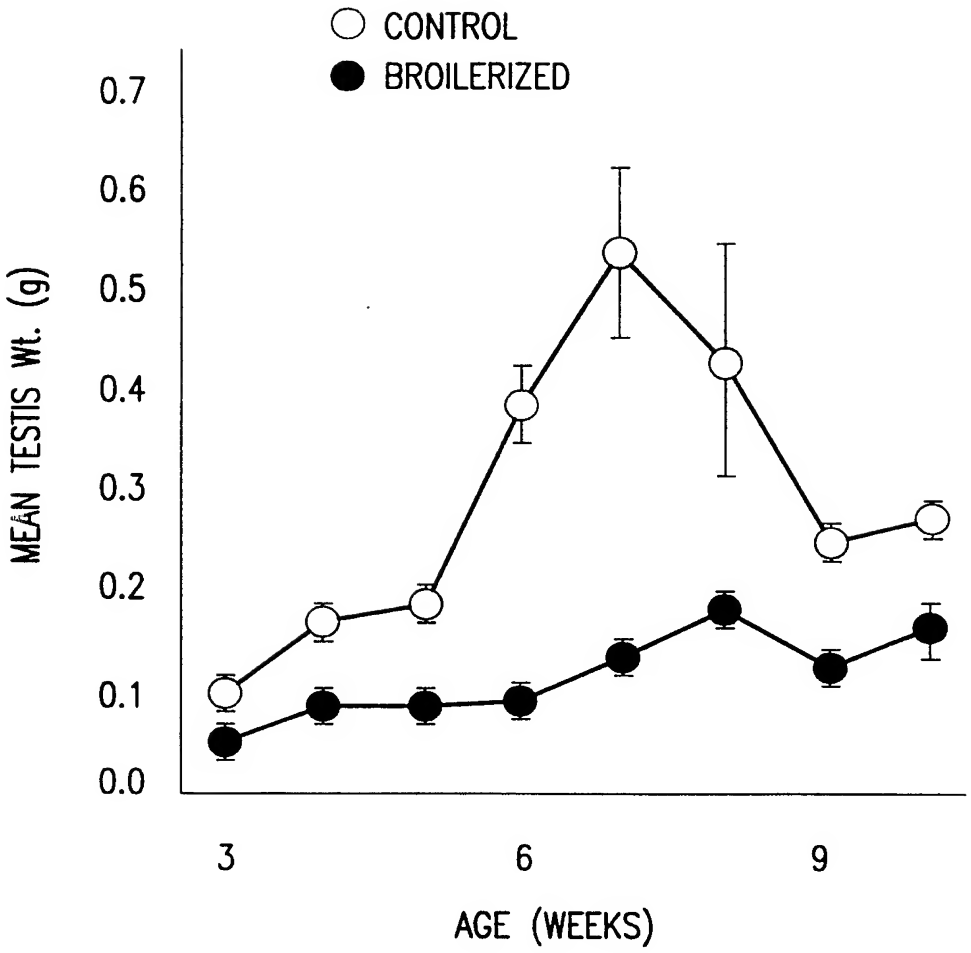

CONTROL
BROILERIZED
MEAN TESTIS Wt. (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
3
6
9
AGE (WEEKS)

FIG.2

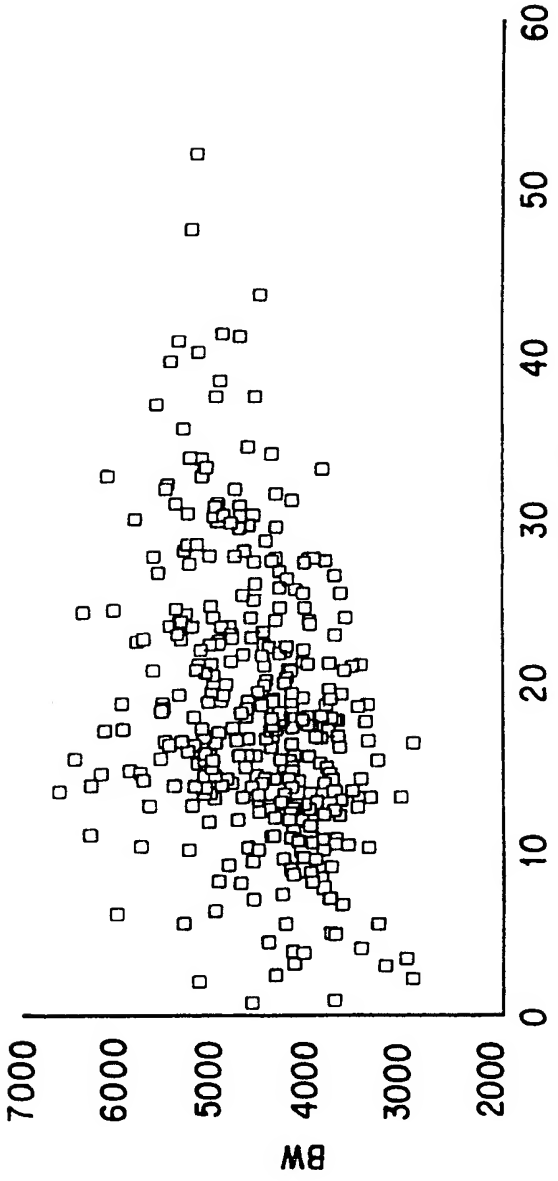


FIG. 3

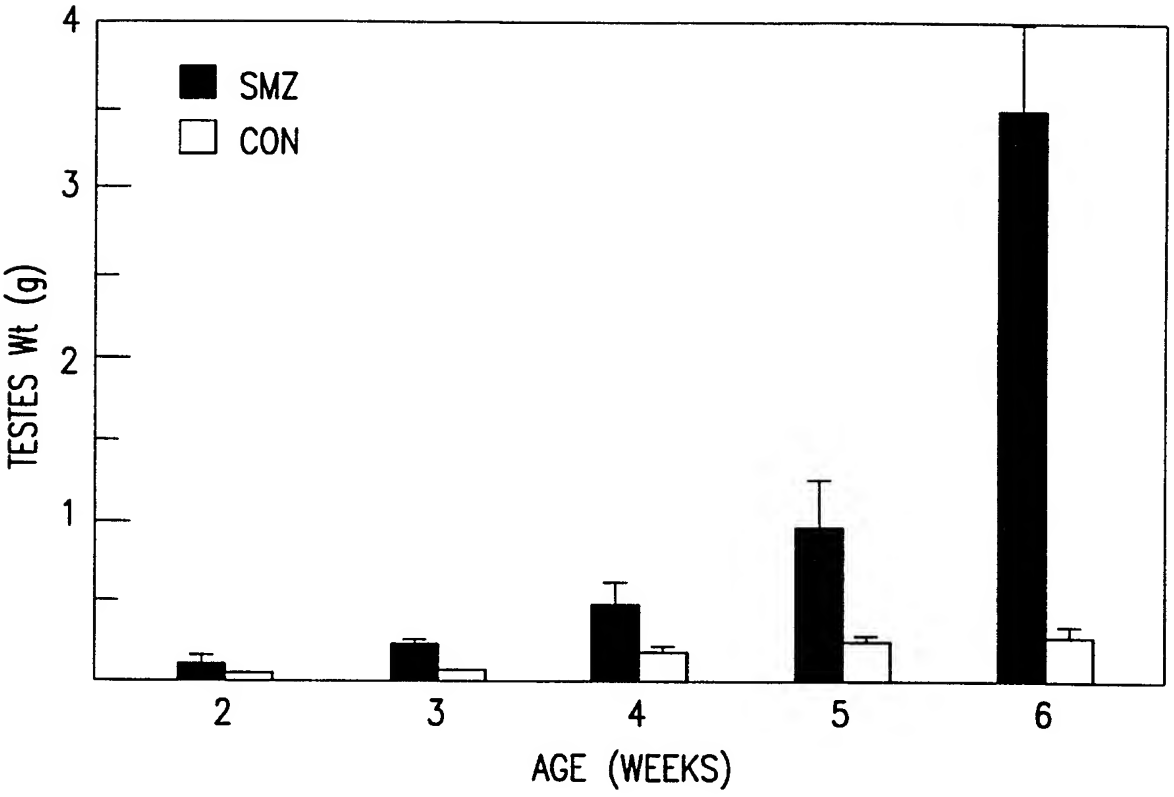


FIG.4

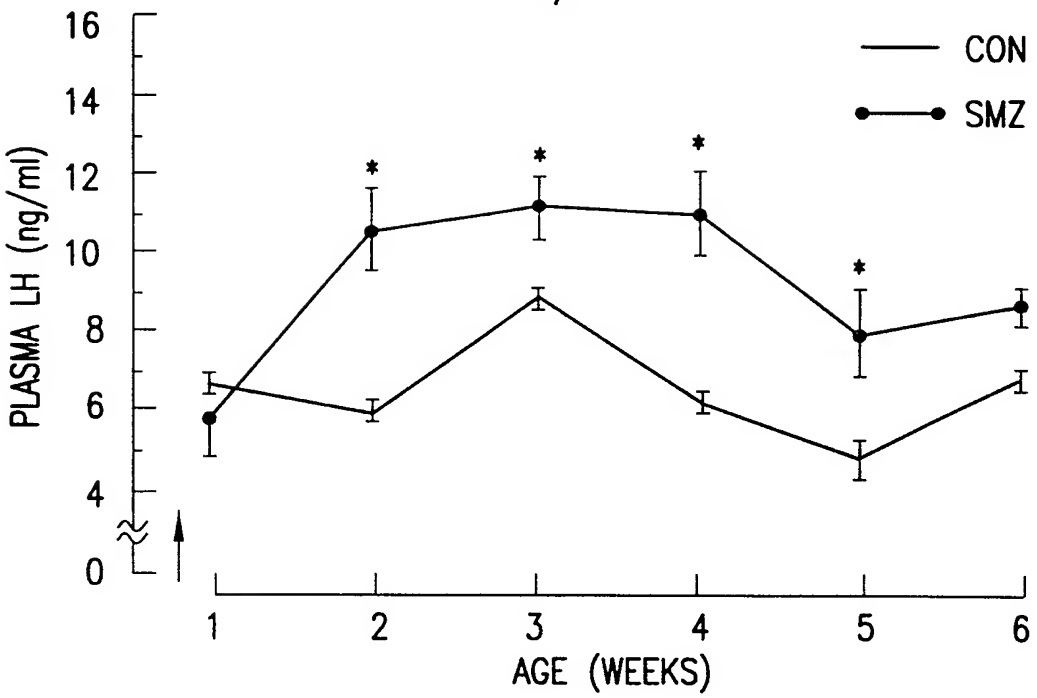

16
14
12
10
8
6
4
0
PLASMA LH (ng/ml)
CON
SMZ
*
*
*
*
1
2
3
4
5
6
AGE (WEEKS)

FIG.5A

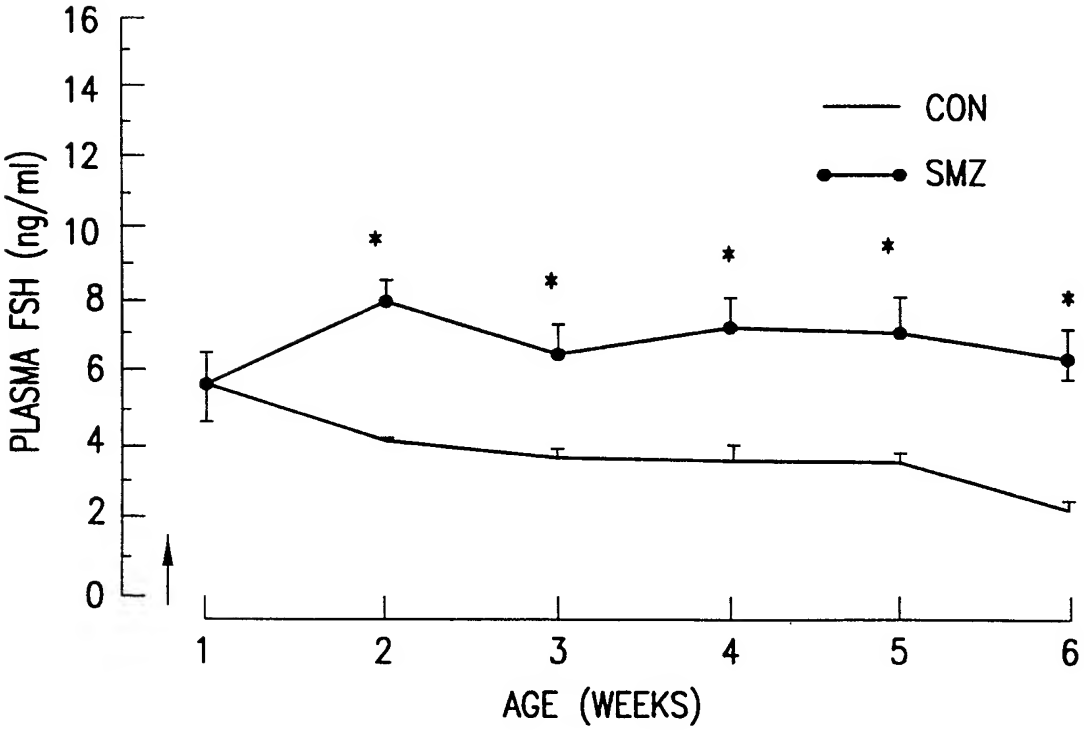

16
14
12
10
8
6
4
2
0
PLASMA FSH (ng/ml)
CON
SMZ
*
*
*
*
*
1
2
3
4
5
6
AGE (WEEKS)

FIG.5B

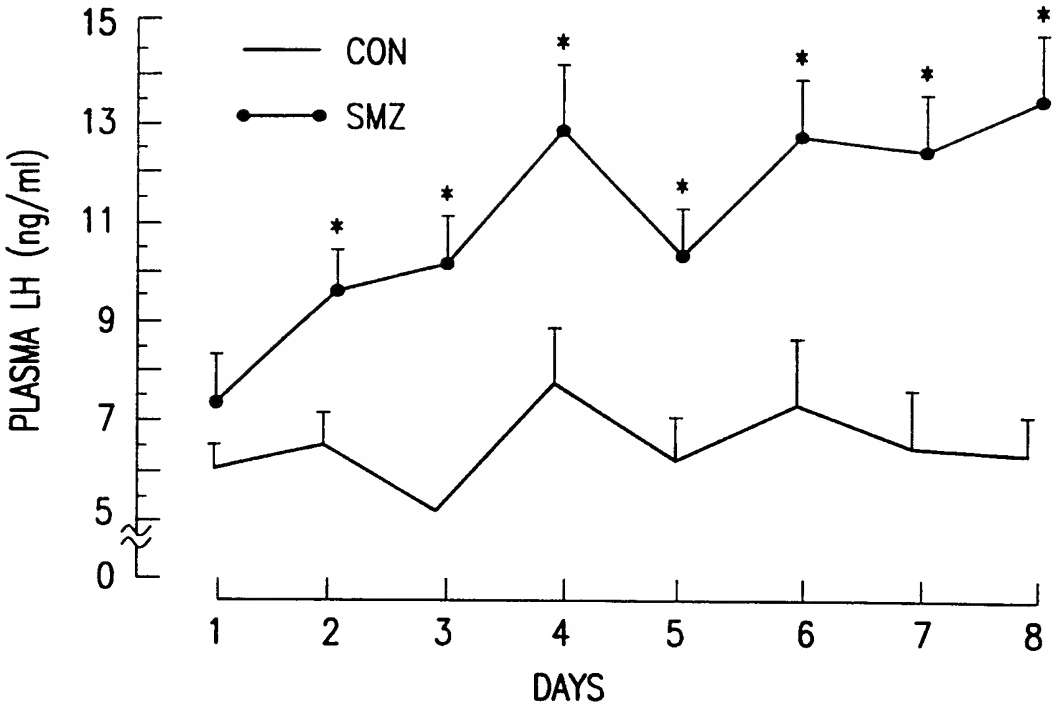


FIG.6

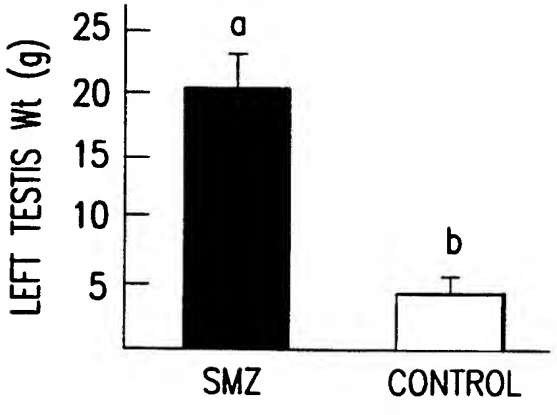


FIG.7A

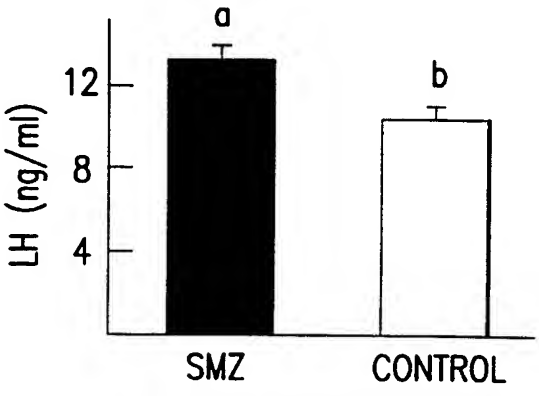


FIG.7B

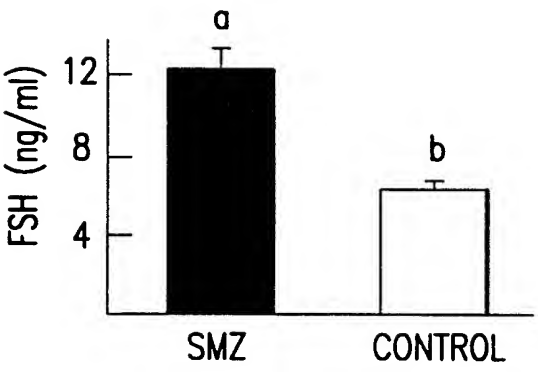


FIG.8A

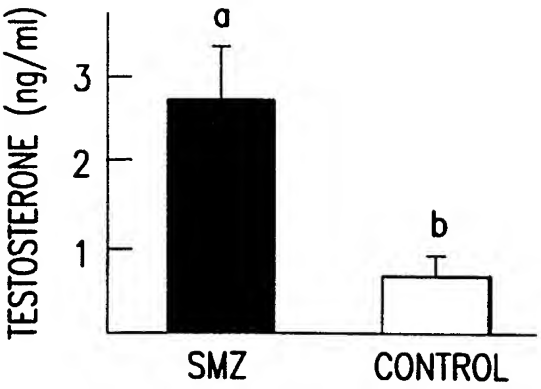


FIG.8B

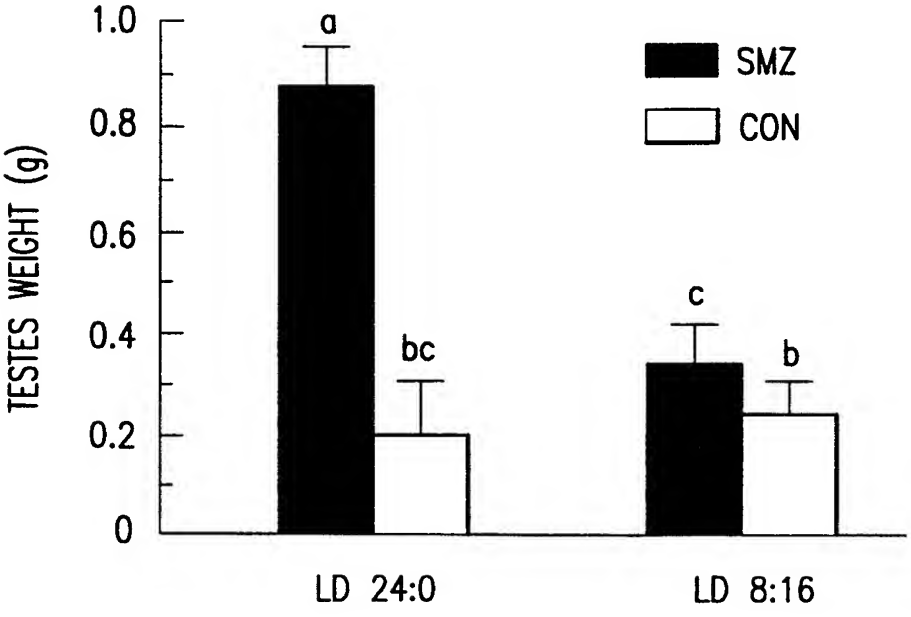


FIG.9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/02520

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A01K 29/00
US CL :119/174; 514/270,580,602, 603,731
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 119/174; 514/270,580,602,603,731

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database WPIDS, London: Derwent Publications Ltd., AN 92-398060/48. DONALDSON, L.E. "Production of super-ovulation in cattle and other farm animals - by administration of follicle stimulating hormone with minor proportion of luteinizing hormone", abstract, NZ 227166 A, 27 September 1993, Vol. 48, see the entire abstract.	1-23
A	Chemical Abstract, Volume 128, abstract No. 113102, WALSH et al, "Effect of Sulfamethazine on Sexual Precocity and Neuropeptide Y Neurons Within the Tuberinfundibular Region of the Chick Brain", Brain Res. Bull. 1997, 44(6), pages 707-713, see entire abstract.	1-23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 APRIL 1999	Date of mailing of the international search report 12MAY1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer FREDERICK KRASS JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★